PATENT
1718-0218PUS1

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT:	MARDH, Goran	CONF:	2500
SERIAL NO.:	10/519,332	GROUP:	1623
FILED:	December 23, 2004	EXAMINER:	KHARE, Devesh

FOR: SYNERGISTIC INTERACTION OF ABACAVIR AND ALOVUDINE

DECLARATION SUBMITTED UNDER 37 C.F.R. § 1.132

Honorable Commissioner
Of Patents and Trademarks
P.O. Box 1450
Alexandria, VA 22313-1450

May 29, 2007

Sir:

I, Dr. Lotta Vrang, Director of Bioscience at Medivir, Sweden, do hereby declare the following:

I have attached a copy of my curriculum vitae to this Declaration.

I am Director of Bioscience and have worked in the virology field for 30 years at the Karolinska Institute, Astra and Medivir.

I am familiar with the above referenced patent application, as well as the development, usages and properties of antiviral drugs, compounds and compositions.

I have read and understand the subject matter of the Office Action of November 28, 2006.

The following comments are offered in support of the patentability of the instant invention.

Claim 1 is currently restricted to combinations with a weight ratio of 0.5 - 5 alovudine : 300 - 800 abacavir. In molar terms this means that the minimum ratio of alovudine to abacavir is 1:51. The significance of this high ratio is explained below.

The Examiner states that, based on the teachings in Margolis to combine abacavir (a guanine nucleoside) with a pyrimidine nucleoside, and the teachings in Harmenberg to combine alovudine (a pyrimidine nucleoside) with a guanine nucleoside, it would have been obvious to the skilled person to prepare a pharmaceutical composition comprising a combination of abacavir (a guanine nucleoside) and alovudine (a pyrimidine nucleoside). However a more comprehensive analysis of Harmenberg leads to the conclusion that it does not motivate the combination of alovudine and an alternative guanosine nucleoside.

It is noted that all the experimental results in Harmenberg are based on the combination of alovudine and the pyrimidine nucleoside, zidovudine. While Harmenberg refers to the purine nucleside ddI, as shown in Table 1 of the present application the combination of ddI and alovudine is markedly less synergistic than alovudine in combination with abacavir, which is the combination being claimed. Note also that Harmenberg, for example at col 3, lines 43-49 stresses that for a synergistic effect, the 3'-fluorinated nucleoside such as alovudine should be administered approximately equimolar with the further nucleoside, with an outside boundary of 1:50 in molar terms. An equimolar formulation is a significantly different regime from the 0.5 - 5 mg alovudine to 300-800 abacavir that is claimed. The upper boundary of the alovudine range (5 mg) and the lower boundary of the abacavir range (300 mg) corresponds to a minimum ratio of 1: 51 in molar terms, based on a molecular weight of alovudine of 244 and abacavir 288.

If the skilled addressee was to endeavor to extend the teachings of Harmenberg to guanosine nucleosides, then even at Harmenberg's extreme limit of 1:50 coming nearest to the presently claimed ranges, the performance of the alovudine/ddG combination as proposed by the Examiner would lead away from the presently claimed combination. In particular in order to investigate whether a synergistic effect could be seen in a combination of alovudine and ddG as indicated in the Harmenberg patent, a study would be performed to evaluate the Combination Index value of alovudine and ddG. This study was conducted.

First, the ED and CI values were calculated as described in WO2004/002433, using a 1:50 ratio of alovudine and ddG. The results are summarized in the table below.

Combination	Molar ratio	Combination Index		
		50% inhibition	75% inhibition	90% inhibition
alovudine+ddG	1:50	1.14	1.59	2.23

These results show an CI above 1 for all three end points measured, namely EC_{50}, EC_{75} and EC_{90}, which should be compared with the alovudine/abacavir combination in table 1 which shows an CI well below 1.

Based on the results of this study, I believe that the skilled practitioner, on reading the Harmenberg patent and carrying out a preliminary trial with an alovudine/ddG combination would not be tempted to extend the teachings of Harmenberg to another guanosine nucleoside.

The undersigned hereby declares that all statements made herein based upon knowledge are true, and that all statements made based upon information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATED: 29 May 2007

Dr. Lotta Vrang

CURRICULUM VITAE

Lotta Vrang
Täppgatan 6
151 33 Södertälje

Phone +46 8 54683160 (work), +46 8 55086915 (home)
E-mail: Lotta.Vrang@medivir.se

Education:

PhD, 1988
Department of Virology, Karolinska Institute, Stockholm "Studies on inhibitors of immunodeficiency virus reverse transcriptase"
Biomedical Scientist 1973

Work experience:

2000-present	Director of Bioscience Medivir AB, Huddinge, Sweden
1988-2000	Group leader, Senior Research Scientist – Bioscience Medivir AB, Huddinge, Sweden
1976-1988	Research Scientist, Anti-viral Chemotherapy Astra ALAB, AB, Södertälje, Sweden
1973-1976	Biomedical Scientist Department of Tumour Biology, Karolinska Institute, Stockholm

Publications:

1: Thorstensson F, Wangsell F, Kvarnstrom I, Vrang L, Hamelink E, Jansson K, Hallberg A, Rosenquist S, Samuelsson B. Synthesis of novel potent hepatitis C virus NS3 protease inhibitors: discovery of 4-hydroxy-cyclopent-2-ene-1,2-dicarboxylic acid as a N-acyl-L-hydroxyproline bioisostere.
Bioorg Med Chem. 2007 Jan 15;15(2):827-38. Epub 2006 Oct 25.

2: Johansson PO, Back M, Kvarnstrom I, Jansson K, Vrang L, Hamelink E, Hallberg A, Rosenquist A, Samuelsson B. Potent inhibitors of the hepatitis C virus NS3 protease: use of a novel P2 cyclopentane-derived template. Bioorg Med Chem. 2006 Aug 1;14(15):5136-51. Epub 2006 May 3.

3: Wannberg J, Sabnis YA, Vrang L, Samuelsson B, Karlen A, Hallberg A, Larhed M. A new structural theme in C2-symmetric HIV-1 protease inhibitors: ortho-substituted P1/P1' side chains. Bioorg Med Chem. 2006 Aug 1;14(15):5303-15. Epub 2006 Apr 18.

4: Muthas D, Noteberg D, Sabnis YA, Hamelink E, Vrang L, Samuelsson B, Karlen A, Hallberg A. Synthesis, biological evaluation, and modeling studies of inhibitors aimed at the malarial proteases plasmepsins I and II. Bioorg Med Chem. 2005 Sep 15;13(18):5371-90.

5: Wannberg J, Kaiser NF, Vrang L, Samuelsson B, Larhed M, Hallberg A. High-speed synthesis of potent C2-symmetric HIV-1 protease inhibitors by in-situ aminocarbonylations. J Comb Chem. 2005 Jul-Aug;7(4):611-7.

6: Johansson PO, Lindberg J, Blackman MJ, Kvarnstrom I, Vrang L, Hamelink E, Hallberg A, Rosenquist A, Samuelsson B. Design and synthesis of potent inhibitors of plasmepsin I and II: X-ray crystal structure of inhibitor in complex with plasmepsin II. J Med Chem. 2005 Jun 30;48(13):4400-9.

7: Ax A, Schaal W, Vrang L, Samuelsson B, Hallberg A, Karlen A. Cyclic sulfamide HIV-1 protease inhibitors, with sidechains spanning from P2/P2' to P1/P1'. Bioorg Med Chem. 2005 Feb 1;13(3):755-64.

8: Lindberg J, Pyring D, Lowgren S, Rosenquist A, Zuccarello G, Kvarnstrom I, Zhang H, Vrang L, Classon B, Hallberg A, Samuelsson B, Unge T. Symmetric fluoro-substituted diol-based HIV protease inhibitors. Ortho-fluorinated and meta-fluorinated P1/P1'-benzyloxy side groups significantly improve the antiviral activity and preserve binding efficacy. Eur J Biochem. 2004 Nov;271(22):4594-602.

9: Shuman CF, Vrang L, Danielson UH. Improved structure-activity relationship analysis of HIV-1 protease inhibitors using interaction kinetic data. J Med Chem. 2004 Nov 18;47(24):5953-61.

10: Johansson PO, Chen Y, Belfrage AK, Blackman MJ, Kvarnstrom I, Jansson K, Vrang L, Hamelink E, Hallberg A, Rosenquist A, Samuelsson B. Design and synthesis of potent inhibitors of the malaria aspartyl proteases plasmepsin I and II. Use of solid-phase synthesis to explore novel statine motifs. J Med Chem. 2004 Jun 17;47(13):3353-66.

11: Dahlgren A, Kvarnstrom I, Vrang L, Hamelink E, Hallberg A, Rosenquist A, Samuelsson B. New inhibitors of the malaria aspartyl proteases plasmepsin I and II. Bioorg Med Chem. 2003 Aug 5;11(16):3423-37.

12: Noteberg D, Schaal W, Hamelink E, Vrang L, Larhed M. High-speed optimization of inhibitors of the malarial proteases plasmepsin I and II. J Comb Chem. 2003 Jul-Aug;5(4):456-64.

13: Andersson HO, Fridborg K, Lowgren S, Alterman M, Muhlman A, Bjorsne M, Garg N, Kvarnstrom I, Schaal W, Classon B, Karlen A, Danielsson UH, Ahlsen G, Nillroth U, Vrang L, Oberg B, Samuelsson B, Hallberg A, Unge T. Optimization of P1-P3 groups in symmetric and asymmetric HIV-1 protease inhibitors. Eur J Biochem. 2003 Apr;270(8):1746-58.

14: Oscarsson K, Oscarson S, Vrang L, Hamelink E, Hallberg A, Samuelsson B. New potent C2-symmetric malaria plasmepsin I and II inhibitors. Bioorg Med Chem. 2003 Apr 3;11(7):1235-46.

15: Dahlgren A, Kvarnstrom I, Vrang L, Hamelink E, Hallberg A, Rosenquist A, Samuelsson B. Solid-phase library synthesis of reversed-statine type inhibitors of the malarial aspartyl proteases plasmepsin I and II. Bioorg Med Chem. 2003 Mar 20;11(6):827-41.

16: Noteberg D, Hamelink E, Hulten J, Wahlgren M, Vrang L, Samuelsson B, Hallberg A. Design and synthesis of plasmepsin I and plasmepsin II inhibitors with activity in Plasmodium falciparum-infected cultured human erythrocytes. J Med Chem. 2003 Feb 27;46(5):734-46.

17: Pyring D, Lindberg J, Rosenquist A, Zuccarello G, Kvarnstrom I, Zhang H, Vrang L, Unge T, Classon B, Hallberg A, Samuelsson B. Design and synthesis of potent C(2)-symmetric diol-based HIV-1 protease inhibitors: effects of fluoro substitution. J Med Chem. 2001 Sep 13;44(19):3083-91.

18: Kim EY, Vrang L, Oberg B, Merigan TC. Anti-HIV type 1 activity of 3'-fluoro-3'-deoxythymidine for several different multidrug-resistant mutants. AIDS Res Hum Retroviruses. 2001 Mar 20;17(5):401-7.

19: Hamalainen MD, Markgren PO, Schaal W, Karlen A, Classon B, Vrang L, Samuelsson B, Hallberg A, Danielson UH. Characterization of a set of HIV-1 protease inhibitors using binding kinetics data from a biosensor-based screen. J Biomol Screen. 2000 Oct;5(5):353-60.

20: Hogberg M, Engelhardt P, Vrang L, Zhang H. Bioisosteric modification of PETT-HIV-1 RT-inhibitors: synthesis and biological evaluation. Bioorg Med Chem Lett. 2000 Feb 7;10(3):265-8.

21: Hogberg M, Sahlberg C, Engelhardt P, Noreen R, Kangasmetsa J, Johansson NG, Oberg B, Vrang L, Zhang H, Sahlberg BL, Unge T, Lovgren S, Fridborg K, Backbro K. Related Articles, Taxonomy via GenBank, Protein, Structure, 3D Domains, LinkOut
Urea-PETT compounds as a new class of HIV-1 reverse transcriptase inhibitors. 3. Synthesis and further structure-activity relationship studies of PETT analogues J Med Chem. 2000 Jan 27;43(2):304.

22: Hogberg M, Sahlberg C, Engelhardt P, Noreen R, Kangasmetsa J, Johansson NG, Oberg B, Vrang L, Zhang H, Sahlberg BL, Unge T, Lovgren S, Fridborg K, Backbro K. Urea-PETT compounds as a new class of HIV-1 reverse transcriptase inhibitors. 3. Synthesis and further structure-activity relationship studies of PETT analogues. J Med Chem. 1999 Oct 7;42(20):4150-60.

23: Alterman M, Andersson HO, Garg N, Ahlsen G, Lovgren S, Classon B, Danielson UH, Kvarnstrom I, Vrang L, Unge T, Samuelsson B, Hallberg A. Design and fast synthesis of C-terminal duplicated potent C(2)-symmetric P1/P1'-modified HIV-1 protease inhibitors. J Med Chem. 1999 Sep 23;42(19):3835-44.

24: Shao X, Rytting AS, Ekstrand DH, Vrang L, Kallander CF, Gronowitz JS. Colorimetric assays for evaluation of the mode of action of human immunodeficiency virus type 1 non-nucleoside reverse transcriptase inhibitors. Antivir Chem Chemother. 1998 Mar;9(2):167-76.

25: Sahlberg C, Noreen R, Engelhardt P, Hogberg M, Kangasmetsa J, Vrang L, Zhang H. Synthesis and anti-HIV activities of urea-PETT analogs belonging to a new class of potent non-nucleoside HIV-1 reverse transcriptase inhibitors. Bioorg Med Chem Lett. 1998 Jun 16;8(12):1511-6.

26: Mauldin SC, Paget CJ Jr, Jones CD, Colacino JM, Baxter AJ, Staschke KA, Johansson NG, Vrang L. Synthesis and antiviral activity of prodrugs of the nucleoside 1-[2',3'-dideoxy-3'-C-(hydroxymethyl)-beta-D-erythropentofuranosyl] cytosine. Bioorg Med Chem. 1998 May;6(5):577-85.

27: Nillroth U, Vrang L, Markgren PO, Hulten J, Hallberg A, Danielson UH. Human immunodeficiency virus type 1 proteinase resistance to symmetric cyclic urea inhibitor analogs. Antimicrob Agents Chemother. 1997 Nov;41(11):2383-8.

28: Bottiger D, Johansson NG, Samuelsson B, Zhang H, Putkonen P, Vrang L, Oberg B. Prevention of simian immunodeficiency virus, SIVsm, or HIV-2 infection in cynomolgus monkeys by pre- and postexposure administration of BEA-005. AIDS. 1997 Feb;11(2):157-62.

29: Cantrell AS, Engelhardt P, Hogberg M, Jaskunas SR, Johansson NG, Jordan CL, Kangasmetsa J, Kinnick MD, Lind P, Morin JM Jr, Muesing MA, Noreen R, Oberg B, Pranc P, Sahlberg C, Ternansky RJ, Vasileff RT, Vrang L,

West SJ, Zhang H. Phenethylthiazolylthiourea (PETT) compounds as a new class of HIV-1 reverse transcriptase inhibitors. 2. Synthesis and further structure-activity relationship studies of PETT analogs. J Med Chem. 1996 Oct 11;39(21):4261-74.

30: Zhang H, Vrang L, Backbro K, Lind P, Sahlberg C, Unge T, Oberg B. Inhibition of human immunodeficiency virus type 1 wild-type and mutant reverse transcriptases by the phenyl ethyl thiazolyl thiourea derivatives trovirdine and MSC-127. Antiviral Res. 1995 Dec;28(4):331-42.

31: Zhang H, Vrang L, Backbro K, Unge T, Noreen R, Oberg B. Enzymatic properties and sensitivity to inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase with Glu-138-->Arg and Tyr-188-->His mutations. Antiviral Res. 1994 May;24(1):43-57.

32: Goobar-Larsson L, Backbro K, Unge T, Bhikhabhai R, Vrang L, Zhang H, Orvell C, Strandberg B, Oberg B. Disruption of a salt bridge between Asp 488 and Lys 465 in HIV-1 reverse transcriptase alters its proteolytic processing and polymerase activity. Virology. 1993 Oct;196(2):731-8.

33: Balzarini J, Karlsson A, Vandamme AM, Perez-Perez MJ, Zhang H, Vrang L, Oberg B, Backbro K, Unge T, San-Felix A, et al. Human immunodeficiency virus type 1 (HIV-1) strains selected for resistance against the HIV-1-specific [2',5'-bis-O-(tert-butyldimethylsilyl)-3'-spiro- 5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide)]-beta-D-pentofurano syl (TSAO) nucleoside analogues retain sensitivity to HIV-1-specific nonnucleoside inhibitors. Proc Natl Acad Sci U S A. 1993 Aug 1;90(15):6952-6.

34: Balzarini J, Karlsson A, Perez-Perez MJ, Vrang L, Walbers J, Zhang H, Oberg B, Vandamme AM, Camarasa MJ, De Clercq E. HIV-1-specific reverse transcriptase inhibitors show differential activity against HIV-1 mutant strains containing different amino acid substitutions in the reverse transcriptase. Virology. 1993 Jan;192(1):246-53.

35: Harmenberg J, Akesson-Johansson A, Vrang L, Cox S. Synergistic inhibition of human immunodeficiency virus replication in vitro by combinations of 3'-azido-3'-deoxythymidine and 3'-fluoro-3'-deoxythymidine. AIDS Res Hum Retroviruses. 1990 Oct;6(10):1197-202.

36: Oberg B, Vrang L. Screening for new agents. Eur J Clin Microbiol Infect Dis. 1990 Jul;9(7):466-71. Review.

37: Koshida R, Vrang L, Gilljam G, Harmenberg J, Oberg B, Wahren B. Inhibition of human immunodeficiency virus in vitro by combinations of 3'-azido-3'-deoxythymidine and foscarnet. Antimicrob Agents Chemother. 1989 May;33(5):778-80.

38: Vrang L, Oberg B, Lower J, Kurth R. Reverse transcriptases from human immunodeficiency virus type 1 (HIV-1), HIV-2, and simian immunodeficiency virus (SIVMAC) are susceptible to inhibition by foscarnet and 3'-azido-3'-deoxythymidine triphosphate. Antimicrob Agents Chemother. 1988 Nov;32(11):1733-4.

39: Eriksson B, Vrang L, Bazin H, Chattopadhyaya J, Oberg B. Different patterns of inhibition of avian myeloblastosis virus reverse transcriptase activity by 3'-azido-3'-deoxythymidine 5'-triphosphate and its threo isomer. Antimicrob Agents Chemother. 1987 Apr;31(4):600-4.

40: Vrang L, Bazin H, Remaud G, Chattopadhyaya J, Oberg B. Inhibition of the reverse transcriptase from HIV by 3'-azido-3'-deoxythymidine triphosphate and its threo analogue. Antiviral Res. 1987 Mar;7(3):139-49.

41: Nyilas A, Vrang L, Drake A, Oberg B, Chattopadhyaya J. The cordycepin analogue of 2,5A and its threo isomer. Chemical synthesis, conformation and biological activity. Acta Chem Scand B. 1986 Sep;40(8):678-88.

42: Vrang L, Oberg B. PPi analogs as inhibitors of human T-lymphotropic virus type III reverse transcriptase. Antimicrob Agents Chemother. 1986 May;29(5):867-72.